Mechanistic modelling of the inhibitory effect of pH on microbial growth

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ABSTRACT

Modelling and simulation of microbial dynamics as a function of processing, transportation and storage conditions is a useful tool to improve microbial food safety and quality. The goal of this research is to improve an existing methodology for building mechanistic predictive models based on the environmental conditions. The effect of environmental conditions on microbial dynamics is often described by combining the separate effects in a multiplicative way (gamma concept). This idea was extended further in this work by including the effects of the lag and stationary growth phases on microbial growth rate as independent gamma factors. A mechanistic description of the stationary phase as a function of pH was included, based on a novel class of models that consider product inhibition. Experimental results on *Escherichia coli* growth dynamics indicated that also the parameters of the product inhibition equations can be modelled with the gamma approach. This work has extended a modelling methodology, resulting in predictive models that are (i) mechanistically inspired, (ii) easily identifiable with a limited work load and (iii) easily extended to additional environmental conditions.

Keywords: Predictive microbiology, mechanistic model, gamma concept, product inhibition.

1 INTRODUCTION

Food safety can be improved by studying the effect of food processing, transportation and storage conditions on microbial growth. In predictive microbiology, mathematical models are developed to describe the effect of environmental conditions on microbial dynamics, allowing accurate predictions of microbial behaviour during the complete life-cycle of food products. In the field of predictive microbiology, a distinction is made between different types of models: (i) primary models describe a microbial response with time for a single set of environmental conditions and (ii) secondary models quantify the effect of environmental conditions on primary model parameters (Whiting and Buchanan, 1993). A typical microbial growth curve described by a primary model includes the lag, exponential and stationary phase of growth. During the stationary phase, the growth rate decreases due to e.g. depletion of a limiting substrate or production of growth-inhibiting metabolites, until the concentration of cells remains constant. Little attention is paid to the description of the stationary phase of growth in predictive microbiology. Many authors argue that only the lag and exponential phase of growth are of importance since many spoilage and pathogenic microorganisms have already caused spoilage or compromised food safety when reaching the stationary phase. However, the stationary phase provides an indication of growth inhibiting mechanisms and interactions among species (McMeekin, 2013). A better description of the stationary phase will also lead to a better understanding of factors that influence microbial growth. As such, an improved understanding of the stationary phase mechanisms will aid in the development of strategies to extend shelf life. Knowledge of these inhibiting effects is also important in experiments with optical density measurement, since the corresponding cell densities are often much higher than cell concentrations gathered from experiments with viable plate counting, and thus, the

effect of the stationary phase on microbial growth is much more pronounced. Secondary models including the effects of multiple environmental conditions on the maximum specific growth rate are often built by assuming that these different environmental factors have separate effects. This assumption was first introduced by McMeekin et al. (1987) and later formalized as the gamma hypothesis by Zwietering et al. (1993). This hypothesis allows to build models describing the separate effects of environmental conditions on the maximum specific growth rate. The multiplicative combination of these independent gamma factors results in an expression describing the global effect. The advantage of this approach is that the effect of additional environmental conditions can easily be integrated by adding new factors to an existing model. The gamma approach has already been applied successfully in several studies (Wijtzes et al., 1995; Wijtzes et al., 2001; Leroi et al., 2012; Lambert and Bidlas, 2007a; Lambert and Bidlas 2007b). In contrast, other studies suggest that interactions should be considered at stressing conditions (Presser, 1998; Augustin and Carlier, 2000; Le Marc et al., 2002; Coroller et al., 2005; Baka et al., 2013; Akkermans et al. 2017).

The availability of high quality experimental data is decisive for building accurate predictive models, so that an efficient experimental design is essential. During these experiments, it is possible to apply either static or dynamic environmental conditions. With static experiments, a single value for the maximum growth rate can be obtained from every growth curve through independent parameter estimations. These values are subsequently used for the parameter estimation of a secondary model. This approach was for instance implemented by Le Marc et al. (2002) to compose a model based on the gamma concept, which describes the combined effect of temperature, pH and organic acid concentration on the growth rate of *Listeria* spp. Alternatively to static

experiments, dynamic experiments offer the possibility to collect more information from a single growth experiment by applying a range of environmental conditions. These dynamic experiments can be combined with optimal experiment design (OED) techniques. The aim of this technique is to collect an optimal amount of information for either parameter estimation or model discrimination. Dynamic experiments were first combined with OED in the field of predictive microbiology by Versyck et al. (1999). The great potential of the combination of dynamic experiments and OED was later confirmed by e.g. Van Derlinden et al. (2010), who designed a single dynamic growth experiment capable of providing sufficient information for an accurate estimation of the cardinal temperatures and optimal growth rate of *E. coli* K12. However, this technique also has limitations. With dynamic experiments, it is often required to combine data from multiple experiments in a single parameter estimation. This may lead to a large number of parameters to be estimated when examining the effect of multiple environmental conditions on the microbial growth rate. The total number of parameters is also high due to many experimental parameters, such as the initial cell density, which are often unknown and should thus be included in the parameter estimation. In the same way, the maximum cell density from the growth model of Baranyi and Roberts (1994) can be included as a separate parameter for every growth curve since it depends on substrate consumption and metabolite production, which are in turn affected by environmental conditions. However, including this empirical value of the maximum cell density in parameter estimations also has drawbacks. At high cell densities during dynamic experiments, growth can be inhibited by both the environmental conditions that are controlled and the inhibiting effects of the stationary phase (e.g., quorum sensing). During a parameter estimation, these effects are hard to

distinguish, potentially leading to a wrong or inaccurate estimation of the parameters of secondary models.

In this paper, the gamma concept will be extended towards primary models, by including the effects of the lag and stationary phase as gamma factors. A more mechanistic description of the stationary phase, based on product inhibition, will also be included in this model. To this end, static experiments are performed at different pH levels with *Escherichia coli* K12. This microorganism is often used as a surrogate for the corresponding pathogenic species, involved, for instance, in a large outbreak in 2011, with 3,816 human cases and 54 deaths (EFSA and ECDC, 2013). The goal of this work is to develop a general modelling approach to build more mechanistic models, which requires a limited amount of experimental effort. This modelling approach will also contribute to the quality of parameter estimations from dynamic experiments.

2 MATERIALS AND METHODS

2.1 Bacterial strain

Escherichia coli K12 MG1655 (CGSC#6300) was acquired from the *E. coli* Genetic Stock Center at Yale University. A stock culture was stored at -80°C in Brain Hearth Infusion broth (BHI, Oxoid), supplemented with 20% (w/v) glycerol (Acros Organics).

2.2 Inoculum preparation

The inoculum was prepared in a three step procedure: (i) A 10 µL loopful of the stock culture was spread onto a BHI agar plate, (BHIA, BHI supplemented with 14 g/L technical agar nr. 3, Oxoid) and incubated overnight at 37°C. (ii) Then, a single colony was transferred into a 50 mL Erlenmeyer containing 20 mL BHI and stored at 37°C for 9 h. (iii) Finally, 20 µL of the stationary phase culture was inoculated into 20 mL fresh BHI and incubated at 37°C for 17 h. A 1:800 dilution of this preculture was used to inoculate the bioreactors at about 1.1×10^3 CFU/mL (or 7 ln(CFU/mL)). The exact volume of preculture to be added to the bioreactor was calculated for each experiment based on the optical density of a 1:10 dilution of the preculture (reference absorbance 0.130 at 600 nm).

2.3 Experimental method

Experiments were performed in computer controlled bioreactors (BioFlo 3000, New Brunswick Scientific Inc.). The reactor vessel was filled with 3.5 L BHI. Temperature was controlled at 37°C for all experiments. pH was controlled at different constant values (5.0, 6.0, 7.0, 8.0, 8.5 and 9.0) by addition of acid (1 N H_2SO_4, Sigma-Aldrich) or base (1 N KOH, Thermo Fisher Scientific). The reactor was aerated with

filtered air at 2 L/min and stirred at 400 rpm. To avoid foaming, 500 µL of an anti-foaming agent (Y-30 emulsion, Sigma-Aldrich) was added to the bioreactor prior to the experiment. Approximately every hour after inoculation, a sample was taken from the bioreactor and the appropriate dilutions were made in BHI and plated onto BHIA plates using a spiral plater (Eddy Jet, IUL Instruments s.a.). These plates were incubated at 37°C for about 15 h and then colonies were counted to obtain viable cell numbers (CFU/mL). Experiments lasted between 12 and 36 h.

2.4 Models

The primary growth model of Baranyi and Roberts (1994) is often used in predictive microbiology. This model was used here to set a benchmark for the comparison with other models. The equations are written using the natural logarithm of the cell density n [$\ln(\mathrm{CFU/mL})$] and the natural logarithm of the physiological state of the cell q [-]:

$$\frac{dn(t)}{dt} = \mu_{max}(pH) \cdot \left(\frac{1}{\exp(-q(t))+1}\right) \cdot (1 - \exp(n(t) - n_{max})) \quad (1)$$

with $n(t = 0) = n_0$

$$\frac{dq(t)}{dt} = \mu_{max}(pH) \quad (2)$$

with $q(t = 0) = q_0$

where μ_{max} [$1/h$] is the maximum specific growth rate at a given pH value and n_{max} [$\ln(\mathrm{CFU/mL})$] is the maximum cell density. In this work, a novel class of secondary models is built by combining the effect of pH, lag and stationary phase on the growth rate (gamma factors) with a basic primary model. The gamma factor

representing the inhibiting effects of the stationary phase is based on the product inhibition factor of the P-model developed by Van Impe et al. (2005):

$$\frac{dN(t)}{dt} = \mu_{max}(pH) \cdot \left(\frac{Q(t)}{Q(t)+1}\right) \cdot \left(1 - \frac{P(t)}{K_P}\right) \cdot N(t) \quad (3)$$

with $N(t = 0) = N_0$

$$\frac{dQ(t)}{dt} = \mu_{max}(pH) \cdot Q(t) \quad (4)$$

with $Q(t = 0) = Q_0$

$$\frac{dP(t)}{dt} = Y_{P/N} \cdot \mu_{max} \cdot \left(\frac{Q(t)}{Q(t)+1}\right) \cdot \left(1 - \frac{P(t)}{K_P}\right) \cdot N(t) \quad (5)$$

with $P(t = 0) = 0$

with N [CFU/mL] the cell density, Q [-] the dimensionless physiological state of the cell, P [M] the concentration of growth inhibiting metabolic products, K_P [M] the maximum concentration of growth inhibiting metabolic products, $Y_{P/N}$ [$M/(CFU/mL)$] the yield of growth inhibiting metabolic products. This model was chosen since it has an equal quality of fit as the widely used Baranyi and Roberts model, but includes a more mechanistic description of the stationary phase through product inhibition. The assumption was made that no inhibiting product is present in the bioreactor at the beginning of the experiment. The model equations as presented above allow the description of the stationary phase as a consequence of product inhibition and different final microbial concentrations (noted as n_{max} in the Baranyi and Roberts model) will be a consequence of this inhibition.

To describe the effect of pH on the maximum specific growth rate $\gamma_{\mu,pH}(pH)$, the Cardinal pH Model (CPM, Rosso et al. 1995) was used:

$$\mu_{max}(pH) = \begin{cases} pH < pH_{min}, 0 \\ pH_{min} < pH < pH_{max}, \mu_{opt} \cdot \gamma_{\mu,pH}(pH) \\ pH > pH_{max}, 0 \end{cases} \quad (6)$$

$$\gamma_{\mu,pH}(pH) = \frac{(pH-pH_{min})\cdot(pH-pH_{max})}{(pH-pH_{min})\cdot(pH-pH_{max})-(pH-pH_{opt})^2} \quad (7)$$

with $\gamma_{\mu,pH}$ [-] the reduction of the maximum specific growth rate due to a deviation from the optimal pH (pH_{opt}) at which the maximum specific growth rate is equal to the optimal growth rate (μ_{opt} [1/h]). pH_{min} and pH_{max} are the limits of the pH range where growth is possible.

2.5 Parameter estimation and confidence intervals

Model parameters were estimated using the *lsqnonlin* routine of the Optimization Toolbox of Matlab version 7.14 (The Mathworks, inc.). A multistart routine was built to help finding the global minimum of the optimization function. This routine was executed at least 25 times for every parameter estimation. The 95% confidence interval of every parameter p_i was calculated based on the Student's t-distribution:

$$\left[p_i \pm t_{0.975,n_s-n_p} \cdot \sqrt{s_{p,i}^2}\right] \quad (8)$$

where n_s and n_p are respectively the number of samples and the number of parameters and thus $n_s - n_p$ is the number of degrees of freedom. $s_{p,i}^2$ is the variance on the parameters and is found as the main diagonal elements of the variance covariance matrix which is approximated as the inverse of the Fisher Information Matrix (F):

$$F = \frac{1}{MSE} \cdot J^T \cdot J \quad \text{with} \quad MSE = \frac{SSE}{n_t - n_p} \quad (9)$$

208 $$V = F^{-1} \quad (10)$$

209 $$s_{p,i}^2 = V(i,i) \quad (11)$$

210

211 with J the Jacobian matrix, MSE the mean sum of squared errors and SSE the sum

212 of squared errors (Walter and Pronzato, 1997).

3 RESULTS AND DISCUSSION

3.1 Effect of pH on the growth of *E. coli* K12

To build the model, a set of six static experiments was performed at different pH values. The results from these experiments are shown in Fig. 1 and Fig. 2. A typical sigmoidal shape is found for all six growth curves. Fig. 1 shows the growth curves in an acidic and neutral environment. These growth curves show that both the maximum specific growth rate and maximum cell density decrease when the environment becomes more acidic with respect to a neutral environment. Fig. 2 shows the growth curves in an alkaline environment. Here, both μ_{max} and n_{max} decrease as the environment becomes more alkaline. The growth curves at pH 7.0 in Fig. 1 and at pH 8.0 in Fig. 2 are very similar regarding both maximum specific growth rate and maximum cell density.

3.2 Parameter estimation with Baranyi and Roberts model

As pointed out by Van Impe et al. (2005), the model of Baranyi and Roberts is widely used due to its (i) easy implementation, (ii) applicability under dynamic conditions, (iii) good quality of fit, and (iv) biological interpretability of most of the model parameters. Accordingly, this model is ideal to set a benchmark for a good model fit of the experimental data shown in Fig. 1 and Fig. 2. In this parameter estimation, different values are estimated for n_0, Q_0, μ_{max} and n_{max} for every experiment, since these parameters are dependent on the experiment and its environmental conditions. The fit of the Baranyi and Roberts model is shown in Fig. 1 and Fig. 2. Parameter estimates are listed in Table 1. In Fig. 3 and Fig. 4, the effect of pH on parameter estimates is illustrated. Since this research focuses on the exponential and stationary phase of the growth curve, the estimated values of μ_{max} and n_{max} are presented here.

Both μ_{max} and n_{max} show lower values in more acidic and alkaline environments compared to neutral conditions. This was also described in the previous section, directly from the growth curves. The MSE of this parameter estimation is 0.037.

3.3 Description of the modelling approach

The first step in composing a primary growth model is to start with the following first order differential equation (or the ln-transformed equivalent):

$$\frac{dN(t)}{dt} = \mu(\cdot) \cdot N(t) \quad \text{with } N(t=0) = N_0 \tag{12}$$

$$\text{or } \frac{dn(t)}{dt} = \mu(\cdot) \quad \text{with } n(t=0) = n_0 \tag{13}$$

in which $\mu(\cdot)$ $[h^{-1}]$ is the specific growth rate. The logarithmic transformation of the cell densities is commonly applied as this transformation stabilises the variance of the experimental measurements (Akkermans et al., 2018) and improves the visualisation of data and model predictions. In this paper, the growth inhibiting effects during the lag and stationary phase are described by the gamma factors $\gamma_l(\cdot)$ and $\gamma_s(\cdot)$, respectively. These gamma factors are combined with $\gamma_{\mu,pH}(pH)$, the gamma factor of the CPM:

$$\mu(\cdot) = \mu_{opt} \cdot \gamma_l(\cdot) \cdot \gamma_{\mu,pH}(pH) \cdot \gamma_s(\cdot) \tag{14}$$

The gamma factor describing the effect of pH on the growth rate $\gamma_{\mu,pH}(pH)$ is expressed using the CPM. The gamma factor, representing the effect of the lag phase on the maximum specific growth rate, is provided with the same structure as the factor

describing the lag phase in the model of Baranyi and Roberts. This mechanistically inspired factor is based on Michaelis-Menten kinetics (Baranyi et al., 1993). Consequently, the following definition for the gamma factor $\gamma_l(\cdot)$ and one additional differential equation are included in the model:

$$\gamma_l(\cdot) = \frac{1}{1+\exp(-q(t))} \tag{15}$$

$$\frac{dq(t)}{dt} = \mu_{max}(pH) \quad \text{with } q(t=0) = q_0 \tag{16}$$

For the dimensionless physiological state of the cell $Q(t)$, the gamma factor is expressed as: $Q(t)/(1+Q(t))$, where $Q(t)$ increases exponentially. This leads to calculations where two very large values are divided by one another, resulting in large numerical errors or even undefined values, when infinity is divided by infinity. These problems are avoided in the above equations by using the natural logarithm of the dimensionless physiological state of the cell $q(t)$.

Next, an equation is required to express the growth inhibiting effects during the stationary phase as a gamma factor. The description of the stationary phase in the Baranyi and Roberts model is based on the logistic model of Verhulst (1838) and is expressed with a maximum carrying capacity, called n_{max} in this instance. However, this maximum carrying capacity is not related to the underlying mechanisms that inhibit growth and is thus an empirical parameter. Van Impe et al. (2005) proposed to use additional equations to describe the consumption of growth-limiting substrates (S-model) and/or the production of growth inhibiting toxic components (P-model). These equations were combined with factors that describe the inhibiting effect of substrate

depletion and toxic products on the growth rate, resulting in a more mechanistic model. This modelling technique was successfully applied in Poschet et al. (2005) to make a more complex model describing coculture growth based on lactic acid formation. The structure of the P-model is given in the materials and methods section. As a case study, this model will be further extended to incorporate the effect of pH, using the gamma approach. The factor describing the effect of the stationary phase can also be defined as a gamma factor:

$$\gamma_s(\cdot) = \left(1 - \frac{P(t)}{K_P}\right) \tag{17}$$

$$\frac{dP(t)}{dt} = Y_{\frac{P}{N}} \cdot \mu(\cdot) \cdot \exp\big(n(t)\big) \tag{18}$$

Since product concentrations were not experimentally determined, these equations are rewritten using the inhibiting product concentration relative to the maximum inhibiting product concentration $p(t)$:

$$\gamma_s(\cdot) = (1 - p(t)) \tag{19}$$

$$\frac{dp(t)}{dt} = \frac{Y_{\frac{P}{N}}}{K_P} \cdot \mu(\cdot) \cdot \exp\big(n(t)\big) \tag{20}$$

with $p(t = 0) = 0$

In the equations above, $Y_{\frac{P}{N}}/K_P$ can be reduced to a single parameter ψ [mL/CFU], which expresses the ratio between the yield of growth inhibiting metabolites $Y_{\frac{P}{N}}$ and the

maximum concentration of a growth inhibiting metabolites K_P. In line with the general gamma approach, the yield parameter ψ could be expressed as:

$$\ln(1/\psi) = \ln\left(1/\psi_{opt}\right) \cdot \gamma_{\psi,pH}(pH) \quad (21)$$

where $\gamma_{\psi,pH}(pH)$ represents the change in production rate of inhibiting product relative to optimal conditions. Similarly to the gamma approach for the maximum specific growth rate, this expression allows several effects to be investigated separately and to be combined afterwards, with or without interactions. As an example, the following expression for $\gamma_{\psi,pH}(pH)$ was tested:

$$\gamma_{\psi,pH}(pH) = \frac{\left(\left(1-e^{\beta\cdot(pH_{min}-pH)}\right)\cdot\left(1-e^{\beta\cdot(pH_{max}-pH)}\right)\right)}{\left(\left(1-e^{\beta\cdot\left(pH_{min}-pH_{opt}\right)}\right)\cdot\left(1-e^{\beta\cdot\left(pH_{max}-pH_{opt}\right)}\right)\right)} \quad (22)$$

with β a dimensionless shape parameter.

3.4 Parameter estimation with the extended P-model

First, a parameter estimation was performed on the full dataset using the model described by Equations 13-20. In this parameter estimation, different values for μ_{max} and ψ were estimated for every experiment. The estimated values of μ_{max} were equal to those estimated with the Baranyi and Roberts model. Moreover, the natural logarithm of the inverted values of ψ ($\ln(1/\psi)$) were equal to the estimates of n_{max}. As such, the parameter estimates are also found in Table 1. This demonstrates that the P-model is structurally equivalent to the Baranyi and Roberts model.

Then, a parameter estimation was performed by including the effect of pH on the microbial growth rate (CPM, Equation 6 and 7) and the yield coefficient (Equation 21 and 22). This model that includes effect of pH is referred to as the extended P-model. The parameter estimation results are shown in Table 2, Fig. 3 and Fig. 4. The growth curves that result from the extended P-model are presented in Fig. 1 and Fig. 2. These figures clearly show the close approximation of the experimental data by the extended P-model. Due to the small deviation between the maximum specific growth rates and the CPM, the MSE is slightly higher at 0.042.

The estimated parameter ψ, or its components $Y_{\frac{P}{N}}$ and K_P, have biological meaning and can also be modelled as a function of other environmental conditions. This approach has already been performed for e.g.: the effect of pH, substrate and oxygen on lactic acid production (Fu and Mathews, 1999); the effect of temperature and pH on citric acid production (Ambati and Ayyanna, 2001); the effect of temperature and pH on bacteriocin production (Messens et al., 2002 and 2003) and the effect of temperature on ethanol fermentation (Phisalaphong et al., 2006). Following the assumption of product inhibition, the gamma factor can also be based on experimental data of the metabolite production. The effect could be explained for instance through the presence of different concentrations of undissociated weak acids and bases at different pH values, since these components are responsible for changes in the intracellular pH (Repaske and Adler 1981).

The modelling methodology proposed here has the following benefits: (i) different experiments are performed for the identification of different model parameters to allow for accurate and unambiguous parameter estimations, (ii) the model structures contain

general information about microbial behaviour, making them suitable for general use (e.g. properties of the Cardinal Temperature Model from Rosso et al. (1993)) and (iii) models containing the effect of many environmental effects can be built with a limited experimental load.

4 CONCLUSION

In this paper, a modelling methodology is presented to build more mechanistic predictive models with a limited experimental load. As a case study, the P-model was extended to describe the effect of pH on the growth of *E. coli* K12. In this model, the inhibiting effects during the lag and stationary phase are also included as gamma factors along with the CPM. The effect of the stationary phase on the growth rate was described by a gamma factor that considers product inhibition. A comparison between the extended P-model and the Baranyi and Roberts model revealed that the first one has a good fitting capacity. Based on the new model structure and results from the parameter estimation, it was concluded that the yield of growth inhibiting metabolites and/or the maximum growth inhibiting metabolite concentration are also a function of pH. Consequently, these parameters can also be described with a gamma approach. The results presented in this research contribute to a global modelling methodology that allows to build accurate predictive models with multiple environmental conditions and requires only a limited experimental effort.

Future research is required to validate the results in a context that is relevant for microbial food safety and quality. As such, further research should include (i) working with a food pathogen or spoiler, (ii) applying food relevant environmental conditions and (iii) monitoring of important substrates and metabolites to be used for mechanistic modelling of the microbial kinetics.

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FIGURE CAPTIONS

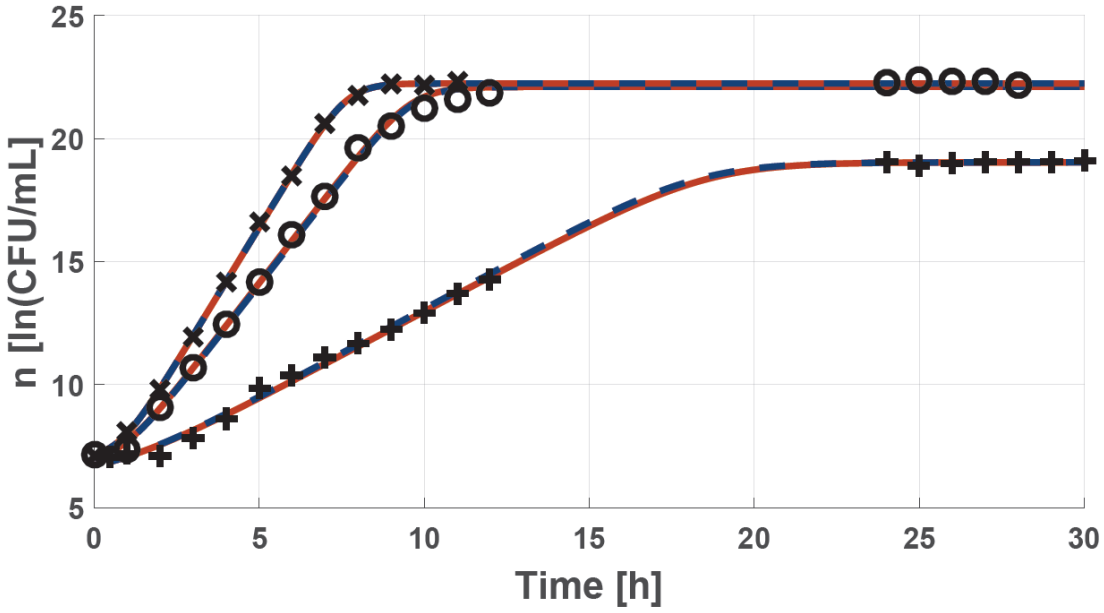
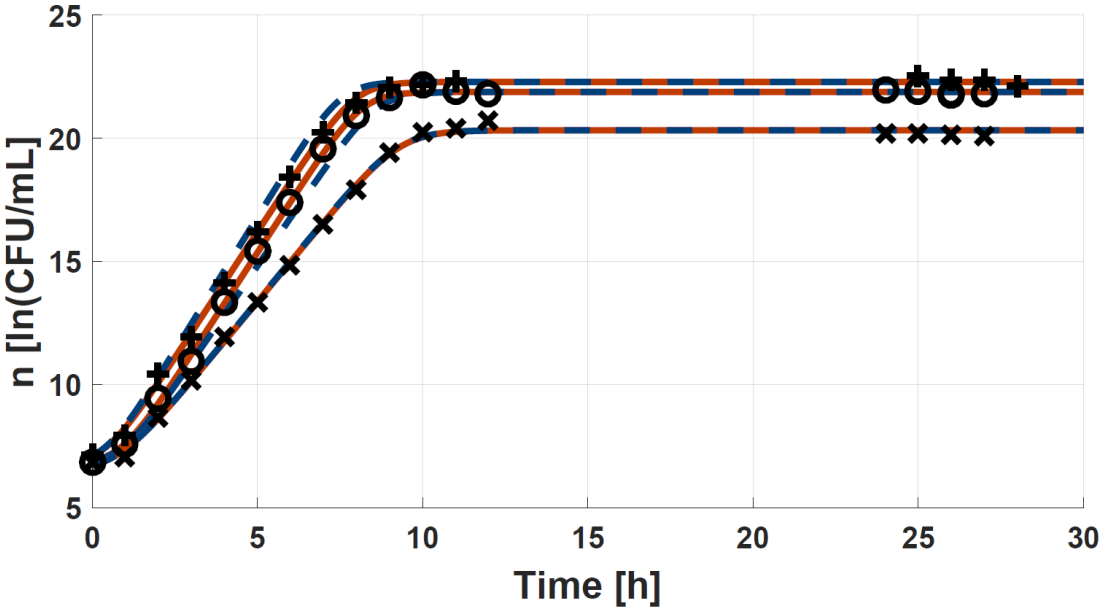


Fig. 1. Growth curves from static experiments with *E. coli* K12 at pH values of 5.0 (+), 6.0 (○) and 7.0 (x) with fits of the Baranyi and Roberts model (—) and the extended P-model (---).



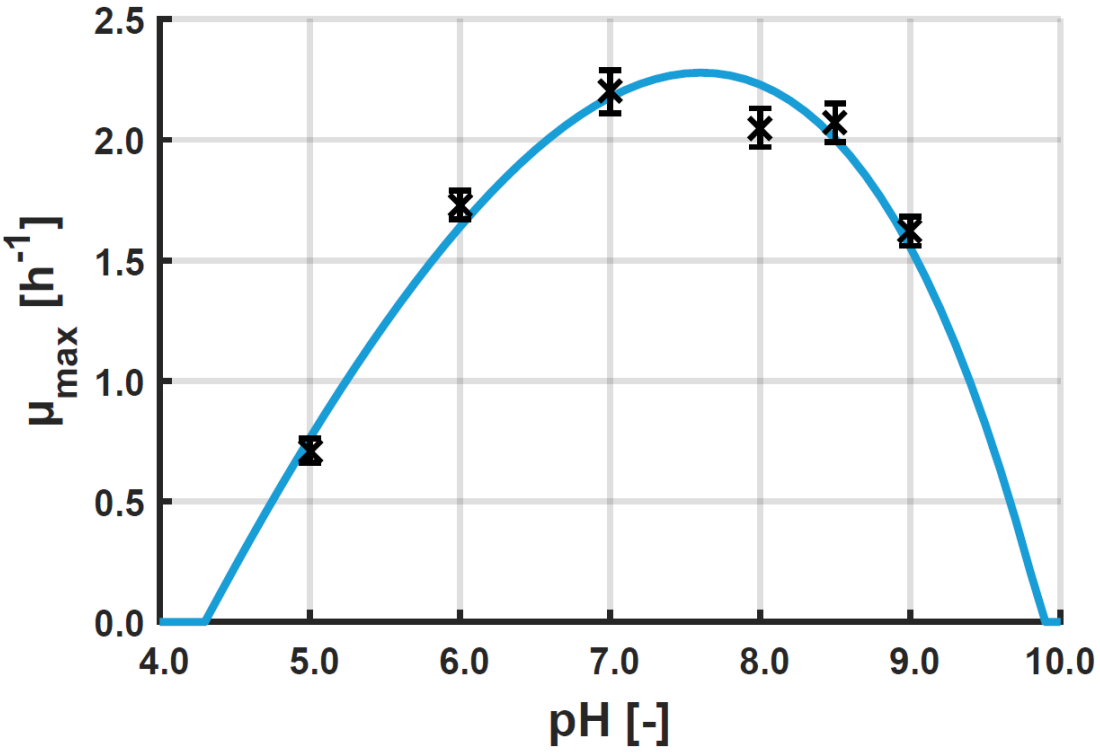
503

504 **Fig. 2.** Growth curves from static experiments with *E. coli* K12 at pH values of 8.0

505 (+), 8.5 (○) and 9.0 (x) with fits of the Baranyi and Roberts model (—) and the extended

506 P-model (---).

507

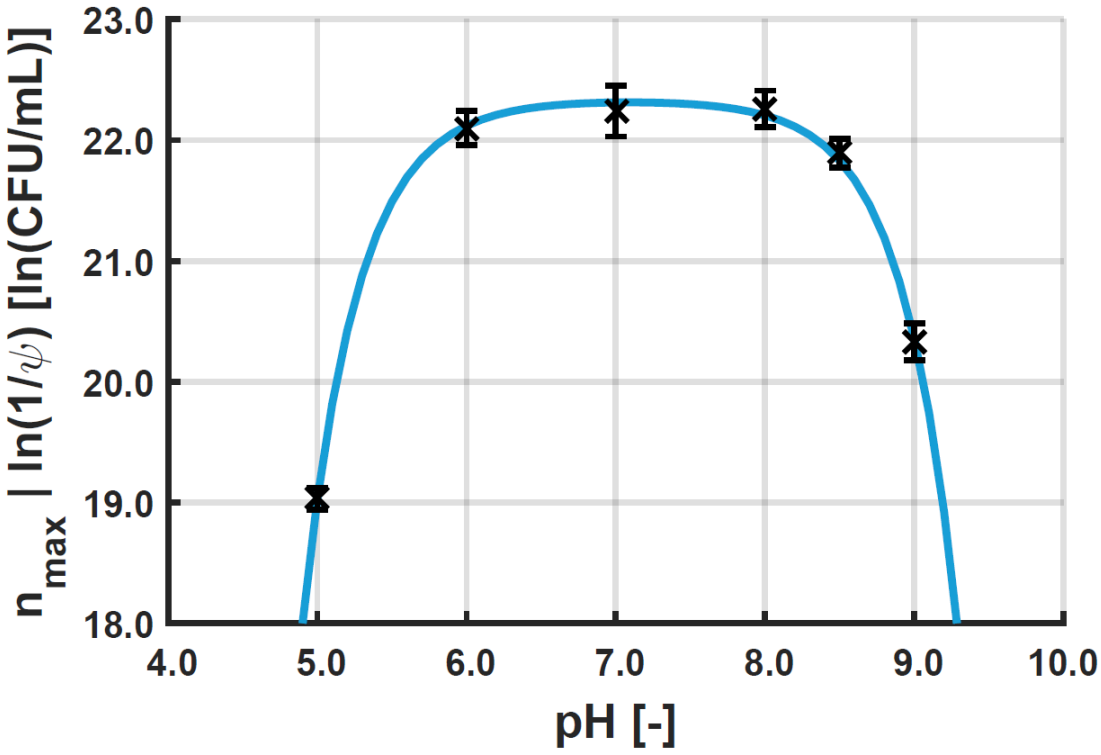


508

509 **Fig. 3.** The effect of pH on the maximum specific growth rate: individual parameter

510 estimates with 95% confidence bounds (x) and the CPM (—).

511



512

513 **Fig. 4.** The effect of pH on the maximum cell density: individual parameter estimates

514 with 95% confidence bounds (x) and the extended P-model (—).

515

516

TABLE CAPTIONS

pH [−]	μ_{max} [1/h]	n_{max} $\left[\ln\left(\frac{CFU}{mL}\right)\right]$
5.0	0.71 ± 0.05	19.03 ± 0.09
6.0	1.73 ± 0.06	22.10 ± 0.14
7.0	2.20 ± 0.09	22.24 ± 0.21
8.0	2.05 ± 0.08	22.26 ± 0.15
8.5	2.07 ± 0.08	21.89 ± 0.12
9.0	1.62 ± 0.07	20.33 ± 0.15

Table 1. Parameter estimates with the width of the 95% confidence intervals for μ_{max} and n_{max} using the model of Baranyi and Roberts.

Parameter	Value
pH_{min} $[-]$	4.30 ± 0.21
pH_{opt} $[-]$	7.61 ± 0.22
pH_{max} $[-]$	9.89 ± 0.26
$\ln(1/\psi_{opt})$ $[-]$	22.30 ± 0.17
β $[-]$	2.70 ± 0.86
μ_{opt} $[1/h]$	2.30 ± 0.16

522

523 **Table 2**. Parameter estimates of the CPM and extended P-model with the width
524 of the 95% confidence intervals.